1. INTRODUCTION

Sleep is a process that plays a very crucial role in the physical and mental well being of every individual. The number of hours spent on sleeping varies among individuals depending upon the age, habitat and the life style. Although sleep requirements varies across the adult human populations, an optimum amount is essential for healthy and productive life. However, over the past few years due to technological advancements and changing life style sleep durations has significantly reduced in human beings all around the world. In a race of development at a rapid scale, every nation has adopted a 24/7 working culture. Youth represents a major percentage of any population and exhibits maximum inclination towards the fast changing society. The outcome of sleep deprivations among modern youth due to societal demands generally remains unrecognized. School – going teenagers representing a huge fraction of any nation's population are more vulnerable to sleep deprivation because of their transient phase. During these periods, not only biological sleep needs increases but their societal interactions also increase enormously. Out of several different factors schools start timings is known to be an important factor to influence sleep behaviors among adolescents as they remain awake even on late nights. Therefore, the present study would deal with to find out the impact of school timings on students.

The hypothesis formulated in the present study is that the students who fail to sleep adequately have elevated stress level and reduced cognitive ability compared to the students who sleep optimally. Hence, the study will also aim at finding out the impact of school timing on stress and cognition of students by using salivary alpha amylase and acetylcholinesterase as markers.

2. A BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD

Sleep: Sleep is a natural biological need which requires to be logistically resupplied like food and water. It is a restorative process characterized by altered consciousness, relatively suspended sensory activity and inactivity of nearly all voluntary muscles. However, it has been shown that some parts of the brain are more active during sleep (Walker and Stickgold, 2006). Although the precise function of the sleep is yet to be ascertained, sleep affects our physical and mental health and is essential for the normal functioning of all the systems of our body including the immune system.

Sleep is not homogenous and is characterized by sleep stages based on electrical brain wave activity (Carney *et al.,* 2005). There are two distinct phases of sleep i.e. rapid eye movement (REM) and non-rapid eye movement (NREM). The stages of sleep were first described in 1937 by Alfred Lee Loomis and his coworkers. NREM is a stage of deep sleep and occurs earlier in the sleep cycle, while REM sleep is an active state which occurs later in the cycle just before natural awakening. REM sleep was discovered as distinct in 1953 by William Dement and Nathaniel Kleitmann who reclassified sleep into four NREM stages and REM.

The criterion of defining sleep into different stages was standardized in 1968 in the "R&K Sleep Scoring Manual" by Allan Rechtschaffen and Anthony Kales. In R&K standard, NREM sleep was divided into four stages with slow wave sleep comprising stage 3 & 4 (Hori *et al.,* 2001). In 2004, the American Academy of Sleep Medicine (AASM) commissioned the AASM Visual Scoring task to review the R & K system. The revised scoring was published in 2007 as "The AASM Manual for the Scoring of Sleep and Associated Events". According to the 2007 AASM standards, NREM consists of three stages: N1, N2, and N3 and a REM stage (Moser *et al.,* 2009).

Sleepiness reflects a basic biological need, analogous to hunger or thirst. (Carskadon et al., 1982). The magnitude of sleepiness is determined by how often and how readily sleep onset occurs, how difficult it is to disrupt sleep and how long sleep endures. (Carskadon and Dement, 1987). The problem of sleepiness may be associated with a range of neurobehavioral complaints including difficulty concentrating, memory lapses, loss of energy, lack of initiative, weariness, fatigue, lethargy and emotional lability (Dinges, 1989, Dahl, 1999).

*School timing and adolescent slee*p: Adolescence is a transitional stage of physical and mental human development generally occurring between puberty and the legal adulthood in which important physical, cognitive, emotional and social changes takes place. Sleep is a primary aspect of adolescent development. The onset of puberty brings a phase shift that causes delayed sleep- wake patterns of an adolescent (Carskadon *et al.,* 1993). Age has been found to be an important factor associated with daytime sleepiness reflecting the effect of pubertal status (Dewald *et al.,* 2010). Earlier research has shown that adolescents require at least as much sleep as they did as children, generally 8 1/2 to 9 1/4 hours each night (Carskadon *et al.,* 1980).

School timings have been shown to play a significant role in Adolescent sleep. Studies related to an adolescent sleep patterns have revealed that they tend to stay up to late night and fail to get adequate sleep of 9 ¼ hours due to early school timings (Price *et al.,* 1978). Inadequate sleep due to delayed bedtime and consumption of caffeinated beverages has been reported to contribute towards reduction in their grade at school (Gibson *et al.,* 2006).

There has been a considerable lack of physical documentation of sleep complaints in hospitalized patients (Meissner *et al.,* 1998; Haponik *et al.,* 1996). A significant relationship found between sleep and school performance of students suggest that sleep habits/history is an important aspect that must be taken into account by the health care practitioners when evaluating adolescents for complaints that may suggest insomnia, delayed sleep phase syndrome or clinical depressions (Wolfson and Carskadon, 2003). The ability to acquire information, retain it and then use it is altered by sleep restriction (Fallone *et al.,* 2001). In a study, conducted by Dexter *et al.* (2003), sleepiness among students from two different schools with different start time (07: 50 AM & 08: 35) was compared. The mean hour slept was found to be 6.88 and 7.07 respectively. In an attempt to normalize the timing of circadian sleep/wake cycle of adolescents by exposure to bright light in the morning,

Hansen *et al.* (2005) reported that both short and long term strategies are required to address this problem in adolescents to improve their health and maximize their school performance. The alertness, mood, and health of students have been found to be enhanced after a modest delay in school start time (Owens *et al.*, 2011; Lufi *et al.*, 2011).

Fredriksen *et al.* (2004) addressed the limitation of such studies by using cross domain latent growth modeling to analyze data from a large (N=2259) sample of adolescents as they progressed through 3 years of middle school. The results were consistent with the previous research. The students who obtained less sleep in 6^{th} grade exhibited lower initial self esteem and grades and higher initial levels of depressive symptoms.

The impact of school timings on school performance is not only limited to the high school students but the middle school students are equally effected (Wolfson *et al.*, 2007).It has also been hypothesized that sleep deprivation in children increases the risk for behavioural symptoms of attention deficit hyperactivity disorder (ADHD).

Earlier school timings which contribute to insufficient sleep in turn leads to increased rate of road accidents among adolescents (Vorona *et al.*, 2011). Therefore, it has been suggested that immediate steps are required to focus on bringing about changes to increase sleep time and reduce the negative impact of sleep deprivation among adolescents (Roberts *et al.*, 2009). Although school education is most important for any sovereign nation, adequate attention has not been paid by countries especially developing countries. Available reports are limited only to the developed nations of the world. In India, which is going to be the richest youth population of the world in coming years, there is no report available except one (Bhatia *et al.*, 2008) on this national important issue.

Stress and sleep quality of adolescents: Sleep plays an important role in maintaining the health of human beings. Adequate sleep ensures proper brain function. Inadequate sleep also results in the hormonal imbalance among children and adolescents (Capaldi, 2005).The stressed students generally do not sleep well thus, contributing to the fact that stress raises the chances of sleep deprivation among adolescents (Mesquita *et al.*, 2009). Poor sleep in children is associated with neuroendocrine alterations (Raikkonen et al., 2010).

Salivary Alpha Amylase as a Biomarker of Stress: Measuring salivary α- amylase levels may help to determine a Soldier's resilience or risk of developing PTSD as part of the research efforts associated with the Comprehensive Soldier Fitness program (Mulrine *et al.*, 2011). Managing stress for student nurse anesthetics also represents an educational concern for anesthesia educators (Mckay *et al*., 2010). A significant relationship has been established between salivary amylase and anxiety levels of student nurse and their performance.

Another stressful task is space flight missions the effect of which has also been studied in astronauts. The 6° head-down tilt (HDT) is established to mimick the low gravity of earth in spaceflights. Stress due to microgravity activates the HPA system and consequently induces significant increases in salivary cortisol and beta-endorphin levels. While increased salivary alpha-amylase levels during simulated microgravity might be due to activation of the sympathetic adrenal medullary system in the beginning and later adapting to conditions of simulated microgravity (Rai and Kaur, 2011). Psychological functioning and concurrent cortisol and sAA activity levels was found to be elevated in adolescents who experienced severe emotional as well as physical setbacks that resulted from significant social and material losses during and following Hurricane Katrina (Vigil *et al.*, 2010).

Nater *et al.* (2004) investigated human sAA levels employing a reliable laboratory stress protocol to examine its reactivity to a brief period of psychosocial stress. sAA exhibits a stable circadian pattern similar to salivary cortisol and it may serve as an easy to use index for SAM activity (Rohleder *et al.*, 2004).

Stress and cognition: The effect of stress on the performance of human could be explained by the field of cognitive psychology. The term cognitive psychology was first used in 1967 by American psychologist Ulrich Neisser in his book *Cognitive Psychology*. According to Neisser, cognition involves all processes by which the sensory input is transformed, reduced, elaborated, stored, recovered, and used. Cognitive psychology is largely an academic discipline and a basic science, concerned primarily with:

(a) Identifying analytically the fundamental components of mental life, such as attention and its allocation, memory systems, problem solving, decision making and the like,
(b) Constructing experimental paradigms to isolate and examine these components in the laboratory, and
(c) Developing theoretical structures that help to make sense of the data collected in these paradigms. But the field is not exclusively academic. General principles have been uncovered over roughly the last forty years of laboratory research on cognition and some of those principles show promise of fruitful application to natural situations, especially in education and training (Bourne and Yaroush, 2003).

Stress affects cognition in a number of ways, acting rapidly via catecholamines and more slowly via glucocorticoids. Prolonged exposure to stress leads to loss of neurons, particularly in the hippocampus (McEwen and Sapolsky, 1995).

Acetylcholinesterase levels and cognition: Acetylcholine is a chemical messenger that transmits a message from the nerve cell to the muscle cell. Acetylcholinesterase (AChE) is an enzyme that catalyses the hydrolysis of neurotransmitters acetylcholine into choline and acetic acid at the nerve synapse and neuromuscular junction. Its activity serves to terminate synaptic transmission.

In 1968, Leuzinger *et al.*, successfully purified and crystallized the enzyme from electric eels at Columbia University, NY. The 3D structure of acetylcholinesterase was first determined in 1991 by Sussman *et al.,* using protein from the Pacific electric ray.

The central cholinergic system is considered to be the most important neurotransmitter involved in regulation of cognitive functions (Francis et al., 1999).

Cognitive decline is a general characteristic of Alzheimer disease. Glycosylation of acetylcholinesterase (AChE) as a diagnostic marker for AD was suggested (Saez-Valero *et al.*, 1997). AChE activity was lower in the CSF of AD patients post mortem as compared to age-matched controls and other cases of dementia. There was a large overlap (40 %) between the groups. However, these authors demonstrated that glycosylated AChE was significantly elevated in AD. Thus lectin-binding analysis of CSF AChE could provide a diagnostic test which is 80 % sensitive and 97 % specific. Many dementias are characterized by loss of cells in the cholinergic basal forebrain (Whitehouse *et al.*, 1981).

Disorders of thyroid functions are also associated with the cognitive decline (Smith *et al.*, 2001) because an extensive inter reliance has been found between thyroid hormone, acetylcholine, nerve growth factor and hippocampal function.. The use of medications with anticholinergic activity also increases the cumulative risk of cognitive impairment and mortality (Fox *et al.*, 2011).

3. OBJECTIVES

The study would be carried out under the following main objectives:

- **I.** To study the impact of school timings on the sleepiness level of students.
- **II.** To study the impact of sleep behavior and the stress on cognitive ability of students.
- **III.** To study the association between acetylcholinesterase levels and the cognition of students

4. PROPOSED METHODOLOGY

The study would target at the higher secondary school students of Chhattisgarh pursuing their studies at different school start timings such as 7:00AM to 8:30AM (Morning shift) and 10:30AM to 12:00 onwards (Dayshift). The study would be executed by following method/procedure:

- **I.** STUDY OF THE SLEEPING BEHAVIOR:

  Sleep behavior and sleepiness level of higher secondary school students will be studied by utilizing Epworth Sleepiness Scale (ESS).

II. SAMPLE COLLECTION:

Saliva samples will be obtained from the subjects in an eppendorf tube. These saliva samples will be stored in the laboratory for further examinations.

III. DETERMINATION OF THE STRESS LEVEL:

Stress level of adolescents would be measured by stress questionnaire designed for students.

IV. DETERMINATION OF THE COGNITIVE ABILITY OF ADOLESCENTS:

Cognitive ability of school going teenagers will be determined using the psychomotor vigilance test of 2-min duration/ Mini-Mental State Examination.

V. ESTIMATION OF THE ACETYLCHOLINESTERASE AND ALPHA AMYLASE ACTIVITY IN SALIVA:

Acetylcholinesterase (AchE) in saliva samples of adolescents will be determined by colorimetric/flourimetric method. Similarly, salivary alpha amylase activity will be determined by using colorimetric method or commercially available assay kit.

VI. STATISTICAL ANALYSIS

The data obtained from the above tests would be analyzed statistically in order to find out the association between different aspects.

5. EXPECTED OUTCOME

Sleep deprivation is becoming a common and serious problem among school going teenagers due to early morning school start time leading to higher stress. Findings have revealed the significance of enzyme salivary alpha amylase and acetylcholinesterase in the determination of stress level and cognitive ability respectively in humans. It is also reported that performance of the students in schools is affected by stress. Therefore the present study focuses mainly on to investigate the relationship between the activity of these two enzymes and the performance of students.

The performance of a student at school gets affected due to the insufficiency of sleep thus giving rise to stress and anxiety. So, measuring the activity of salivary α amylase could prove useful in determining the activation of sympatho-adrenalmedullary axis and hence to understand the extent to which a student is having stress. And as stress is known to interfere with the cognitive ability in humans, it would also be useful to

measure the resilience of students to stressed conditions. Acetyl cholinesterase levels which is known to be important in determining cognitions in humans would be useful in interpreting the outcomes of stress and sleep deprivation.

Thus, findings of this study may be useful for policy makers in deciding proper school timings for better performance with minimum stress. Further, this study may also be helpful in providing proper parental care and attention to those children who are prone to develop depressive symptoms later.

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